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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of: Arthur SCHAFFER, et al.

Serial No. : 09/744,085

Filed : March 26, 2001

For : CONTROLLING STARCH SYNTHESIS

Group Art Unit: 1638

Examiner: David T. Fox

RULE 132 DECLARATION OF ARTHUR SCHAFFER

I, the undersigned, Arthur Schaffer, of 16 Hazayit Street, Hashmonaim, Israel hereby declare as follows:

1. I am the Applicant in U.S. Patent Application Ser. No. 09/744,085, filed March 26, 2001 (hereinafter "the application").

2. I received a Ph.D. in Plant Genetics and Physiology from Rutgers University in 1982 and was a post-doctoral Fellow at the Hebrew University, Faculty of Agriculture, Rehovot, Israel from 1982-1985. Since 1985, I have served as a Research Scientist at ARO, The Volcani Center, Institute of Field and Garden Crops. I have served as Chairman of the Department of Vegetable Crops at ARO since 2000. I have co-authored many articles that have been published in *Plant Physiology*, *Plant Science* and other publications in the field of plant physiology.

3. The claims of the aforesaid patent application, and specifically independent claim 1, stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

4. The Examiner, in the Office Action dated October 3, 2002 (paper no. 15), objected to claim 1, which recited:

A method for controlling starch synthesis in tomatoes comprising:  
providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes; and

selecting individuals of said population that each contain an allele of a gene that increases starch synthesis, said gene originating from said *Lycopersicon* spp.

The Examiner, in his reasons for rejection of claim 1, wrote: "the specification only provides guidance for a plant produced by crossing *L. hirsutum* and *L. esculentum* ... No guidance is given for crossing any other green-fruited tomato species with *L. esculentum* ..."

5. In order to show that the specification provides guidance for crossing other green-fruited tomato species with *L. esculentum*, I have carried out experiments using only the experimental methods listed in the specification. These data show that by providing a population of plants derived from the interspecific cross of an additional green fruited wild tomato species, *Lycopersicon pennellii* with *L. esculentum*, genotypes containing the *L. pennellii* allele for the LS1 of ADPGPPase have increased activity of the enzyme ADPGPPase as well as increased starch content in the young fruit, in a manner similar to that described in the patent using the wild species *L. hirsutum*.

In the new experiments the introgression lines of *L. esculentum* cv. M-82 which contain introgressions of portions of the *L. pennellii* genome, (as described in Eshed and Zamir, 1994, Theoretical Applied Genetics, 88:891-897) were used. Specifically, the introgression line IL1-4, which contains the portion of chromosome 1 from *L. pennellii* harboring the locus for the LS1 gene, was grown alongside the recurrent parent M-82, containing the *L. esculentum* portion of the chromosome 1 and therefore the *L. esculentum* allele for the LS1 gene. The plants were grown (10 of each) in the open field under standard agrotechniques during the spring, 2003 in Gedera, Israel. Six individual fruitlets, approximately 12-14 grams each, were sampled and assayed for starch content and ADPGPPase activity, as described in Schaffer and Petreikov, 1997a,b (Plant Physiology 113:739-746; Physiologia Plantarum 101:800-806) and in the specifications of the patent application.

The results are as follows:

Plant genotype	Fruitlet weight	ADPGPPase Activity	Starch mg/gfw
M-82	13.9 a	104.8 a	9.1 a
M-82 with <i>L. hirsutum</i> LS1	13.3 a	271.2 b	16.5 c
M-82 with <i>L. pennellii</i> LS1	12.3 a	216.3 b	13.3 b

a,b,c indicate statistical significance  $P < 0.5$ .

As can be seen from the data, the *L. pennellii* introgression containing the LS1 allele from *L. pennellii* increases young fruit starch content and ADPGPPase activity in a manner similar to that characterized by the *L. hirsutum* introgression containing the LS1 allele.

Accordingly, increased ADPGPPase activity, which is a limiting factor in starch synthesis in tomato fruits, can be obtained by introgressing the LS1 subunit allele from other green-fruited wild *Lycopersicon* species and is not limited to *L. hirsutum*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and conjecture are thought to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.



Ari Schaffer, Citizen of Israel  
16 Hazayit Street, Hashmonaim, Israel  
July 21, 2003